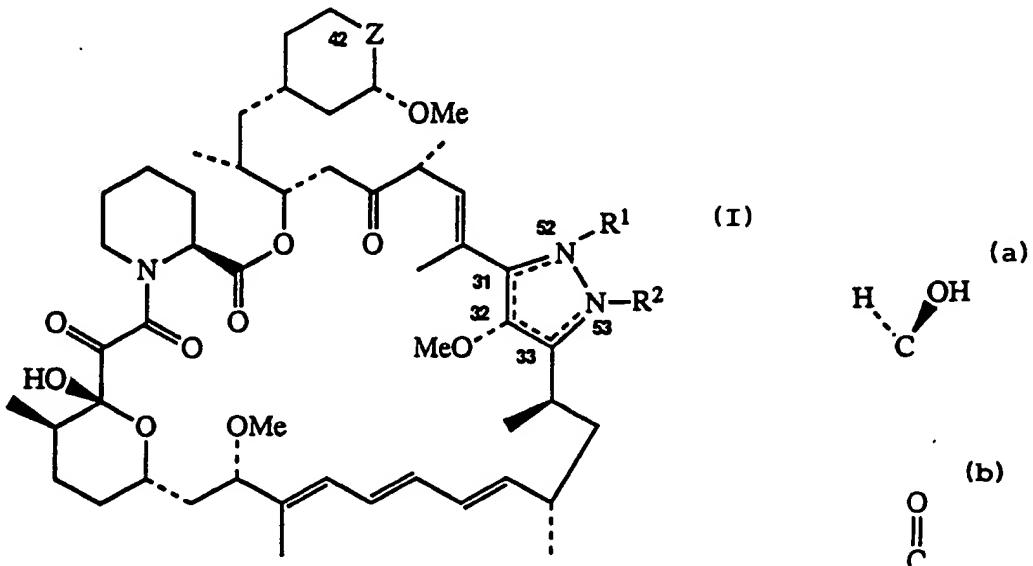




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## (54) Title: RAPAMYCIN PYRAZOLES



## (57) Abstract

A compound of formula (I) wherein Z is (a) or (b); and R<sup>1</sup> is hydrogen, alkyl, or arylalkyl, provided that when R<sup>2</sup> is present, R<sup>1</sup> is absent; R<sup>2</sup> is hydrogen, alkyl, or arylalkyl, provided that when R<sup>1</sup> is present, R<sup>2</sup> is absent; the dotted lines in the pyrazole ring represent double bonds between the 31- and 32-positions and between the 33- and 53-positions when R<sup>1</sup> is present and double bonds between the 31- and 52-positions and between the 32- and 33-positions when R<sup>2</sup> is present; or a pharmaceutically acceptable salt thereof which by virtue of its immunosuppressive activity is useful in treating transplantation rejection, host vs. graft disease, autoimmune diseases, and diseases of inflammation, by virtue of its antifungal activity is useful in treating fungal infections; and by virtue of its antitumor activity is useful in treating tumors. Intermediates possessing a 31,33-β-dicarbonyl moiety that are useful for the same purposes are also described.

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## RAPAMYCIN PYRAZOLES

### BACKGROUND OF THE INVENTION

This invention relates to pyrazoles of rapamycin and intermediates thereof and a method for using them in the treatment of transplantation rejection, host vs. graft disease, autoimmune diseases, diseases of inflammation, tumors, and fungal infections.

Rapamycin is a macrocyclic triene antibiotic produced by Streptomyces hygroscopicus, which was found to have antifungal activity, particularly against 10 Candida albicans, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S.N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Patent 3,929,992; and U.S. Patent 3,993,749].

Rapamycin alone (U.S. Patent 4,885,171) or in combination with picibanil (U.S. Patent 4,401,653) has been shown to have antitumor activity. R. Martel et al. 15 [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

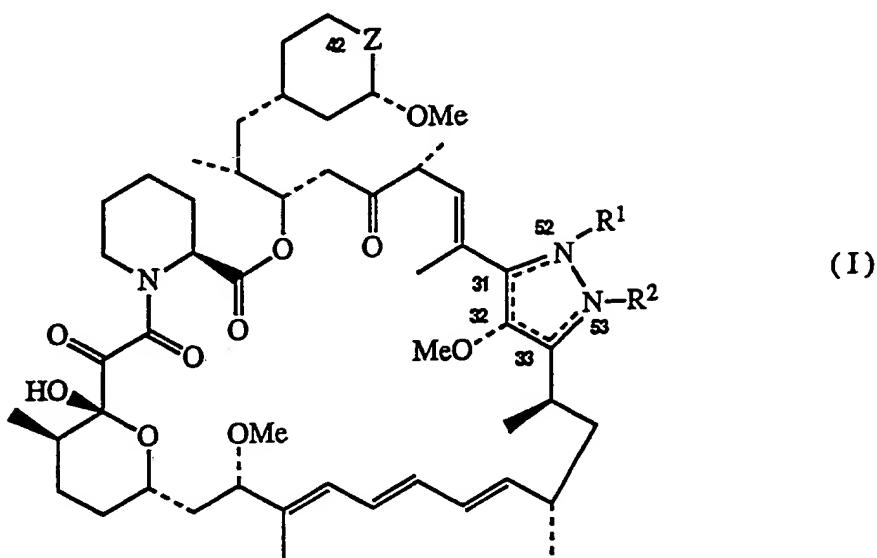
The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 20 3411 (1989). The ability of rapamycin to prolong survival time of organ grafts in histoincompatible rodents was disclosed by Morris. [Med. Sci. Res. 17: 877 (1989)]. Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); and R. Y. Calne et al., Lancet 1183 (1978)].

Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Patent 4,316,885) and used to make water soluble prodrugs of rapamycin (U.S. Patent 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore 30 according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42- positions.

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## DESCRIPTION OF THE INVENTION

This invention provides derivatives of rapamycin which are useful as immunosuppressive, anti-inflammatory, antitumor, and antifungal agents having the structure



5

wherein Z is or ; and

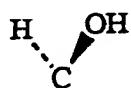
R<sup>1</sup> is hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms,  
provided that when R<sup>2</sup> is present, R<sup>1</sup> is absent;

R<sup>2</sup> is hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms,  
provided that when R<sup>1</sup> is present, R<sup>2</sup> is absent;

10 the dotted lines in the pyrazole ring represent double bonds between the 31- and  
32-positions and between the 33- and 53-positions when R<sup>1</sup> is present  
and double bonds between the 31- and 52-positions and between the 32-  
and 33-positions when R<sup>2</sup> is present;

15 or a pharmaceutically acceptable salt thereof.

Of the compounds, preferred members are those in which Z is



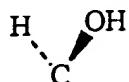
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It is preferred that the aryl portion of the arylalkyl substituent is a phenyl group that is optionally mono-, di-, or tri-substituted with a substituent selected from the group consisting of alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, and carboxy.

The pharmaceutically acceptable salts may be formed from inorganic cations such as sodium, potassium, and the like; mono-, di-, and trialkyl amines of 1-6 carbon atoms, per alkyl group and mono-, di-, and trihydroxyalkyl amines of 1-6 carbon atoms per alkyl group; and organic acids such as acetic, lactic, citric, tartaric, succinic, maleic, malonic, gluconic, and the like.

This invention also provides processes for preparing the pyrazoles of rapamycin and intermediates. The pyrazoles of formula I as defined above are prepared by one of the following

a) reacting 31-oxorapamycin with hydrazine to give a compound of formula I as defined above where Z is

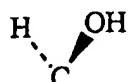


and R<sup>1</sup> or R<sup>2</sup> is hydrogen;

b) reacting a compound of formula I as above wherein R<sup>1</sup> or R<sup>2</sup> is hydrogen with an alkylating agent containing the group R<sup>3</sup> where R<sup>3</sup> is R<sup>1</sup> or R<sup>2</sup> as defined above excepting hydrogen;

or

c) oxidizing a compound of formula I as defined above wherein Z is

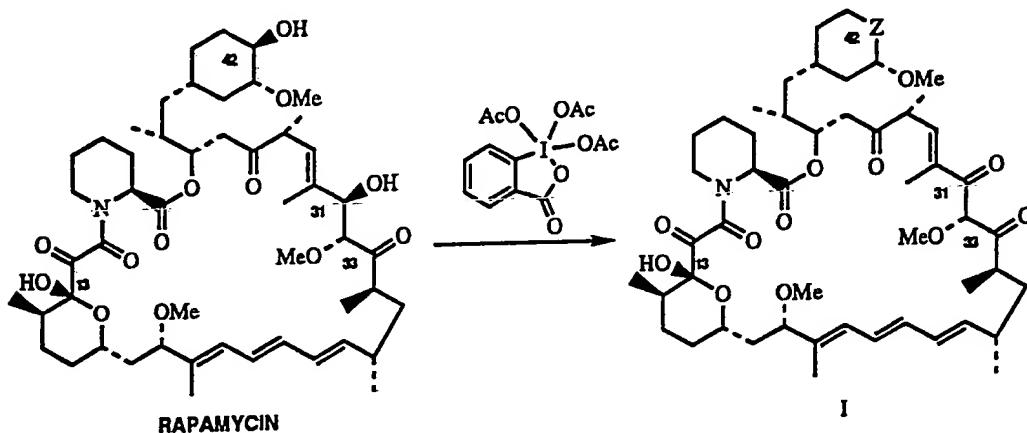


to give a corresponding compound of formula I wherein Z is C=O,

and if desired after any one of steps a), b) or c) isolating the compound of formula I as a pharmaceutically acceptable salt.

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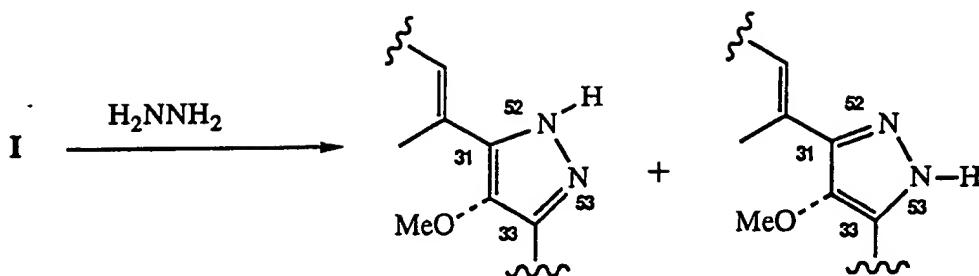
The compounds of this invention can be prepared by oxidizing rapamycin with a periodinane oxidizing reagent such as, Dess-Martin periodinane [J. Org. Chem. 48: 4155 (1983)], to selectively provide either 31-oxorapamycin or 31,42-dioxorapamycin, as shown below.



5. Although oxidation of a secondary alcohol to a ketone can be accomplished by numerous methods that have been described in the literature, selective oxidation of any of the hydroxy functions in polyhydroxylated macrocycles is by no means a trivial undertaking, since in such systems functional group reactivity cannot be readily predicted. [R.B. Woodward et al., J. Am. Chem. Soc. 103, 3215 (1981)]. Additionally, the oxidation of the hydroxyl functions of rapamycin was particularly difficult to achieve, since this polyfunctional macrocyclic lactone is sensitive to many of the conditions and reagents (particularly those containing chromium) which are routinely employed to oxidize other, less sensitive substrates. A method of making 42-oxorapamycin was disclosed in U.S. Patent 5,023,263 using a rhuthenium oxidant; however, the selective oxidation of the 31-position of rapamycin over the oxidation of the 42-position was particularly unexpected in light of U.S. Patent 5,023,263 which teaches the selective oxidation of the 42-position of rapamycin.

20 For the compounds of this invention in which the 42-position has not been oxidized, the pyrazole compounds of this invention can be made from the 31,33- $\beta$ -dicarbonyl moiety by treatment with hydrazine, to provide a mixture of tautomers as shown below.

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Substitution of the pyrazole ring to provide compounds in which R<sup>1</sup> or R<sup>2</sup> is other than hydrogen, can be accomplished by reacting the mixture of tautomeric pyrazole adducts with an alkylating agent, such as an alkyl halide in the presence of an acid scavenger, such as triethylamine or solid potassium carbonate, to provide a mixture of regioisomers that can be separated by conventional methods such as preparative high performance liquid chromatography. [see for example, Pyrazoles, Pyrazolines, Pyrazolidines, Indazoles and Condensed Rings, Wiley ed, 71 (1967)].

For the compounds of this invention in which the 42-position has been oxidized to a ketone, the pyrazole ring is made by first reacting the 31,33-β-dicarbonyl with hydrazine providing the above described pyrazole adduct followed by oxidation of the 42-hydroxyl group with Dess-Martin periodinane or a ruthenium based oxidizing agent. The oxidation step can be carried out either before or after alkylation of the pyrazole nitrogen for compounds in which R<sup>1</sup> or R<sup>2</sup> is other than hydrogen.

The reagents used to prepare the compounds of the invention are commercially available or can be prepared by methods that are disclosed in the literature.

Immunosuppressive activity was evaluated in an in vitro standard pharmacological test procedure to measure lymphocyte proliferation (LAF) and in two in vivo standard pharmacological test procedures. The first in vivo procedure was a popliteal lymph node (PLN) test procedure which measured the effect of compounds of this invention on a mixed lymphocyte reaction and the second in vivo procedure evaluated the survival time of a pinch skin graft.

The comitogen-induced thymocyte proliferation procedure (LAF) was used as an in vitro measure of the immunosuppressive effects of representative compounds. Briefly, cells from the thymus of normal BALB/c mice are cultured for 72 hours with PHA and IL-1 and pulsed with tritiated thymidine during the last six hours. Cells are

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cultured with and without various concentrations of rapamycin, cyclosporin A, or test compound. Cells are harvested and incorporated; radioactivity is determined. Inhibition of lymphoproliferation is assessed in percent change in counts per minute from non-drug treated controls. The results are expressed as the IC<sub>50</sub>, expressed in 5 nM, or as the percent inhibition at 100 nM.

A mixed lymphocyte reaction (MLR) occurs when lymphoid cells from genetically distinct animals are combined in tissue culture. Each stimulates the other to undergo blast transformation which results in increased DNA synthesis that can be quantified by the incorporation of tritiated thymidine. Since stimulating a MLR is a 10 function of disparity at Major Histocompatibility antigens, an *in vivo* popliteal lymph node (PLN) test procedure closely correlates to host vs. graft disease. Briefly, irradiated spleen cells from BALB/c donors are injected into the right hind foot pad of recipient C3H mice. The drug is given daily, i.p. from Day 0 to Day 4. On Day 3 and Day 4, tritiated thymidine is given i.p., b.i.d. On Day 5, the hind popliteal lymph 15 nodes are removed and dissolved, and radioactivity counted. The corresponding left PLN serves as the control for the PLN from the injected hind foot. Percent suppression is calculated using the non-drug treated animals as allogenic control. Rapamycin at a dose of 6 mg/kg, p.o. gave 86% suppression, whereas cyclosporin A at the same dose gave 43% suppression. Results are expressed by the following ratio:

20  $\frac{^3\text{H-PLN cells control C3H mouse} - ^3\text{H-PLN cells rapamycin-treated C3H mouse}}{^3\text{H-PLN cells control C3H mouse} - ^3\text{H-PLN cells test compound-treated C3H mouse}}$

The second *in vivo* test procedure is designed to determine the survival time of 25 pinch skin graft from male DBA/2 donors transplanted to male BALB/c recipients. The method is adapted from Billingham R.E. and Medawar P.B., J. Exp. Biol. 28:385-402, (1951). Briefly, a pinch skin graft from the donor is grafted on the dorsum of the recipient as a homograft, and an autograft is used as control in the same region. The recipients are treated with either varying concentrations of cyclosporin A as test control or the test compound, intraperitoneally. Untreated recipients serve as rejection control. The graft is monitored daily and observations are recorded until the graft becomes dry 30 and forms a blackened scab. This is considered as the rejection day. The mean graft survival time (number of days  $\pm$  S.D.) of the drug treatment group is compared with the control group.

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The following table summarizes the results of a representative compound of this invention (Example 3) and the two 31,33- $\beta$ -dicarbonyl intermediates (Examples 1 and 2) in these three standard test procedures.

TABLE 1\*

		LAF		PLN	Skin Graft (days $\pm$ SD)
	<u>Compound</u>	<u>IC<sub>50</sub> (nM)</u>	<u>(100 nM)<sup>+</sup></u>	<u>(ratio)</u>	
5	Example 1	84.9 <sup>++</sup>	41	**	9.16 $\pm$ 0.47
	Example 2		4	**	**
10	Example 3	205.3 <sup>++</sup>		0.52	**
	Rapamycin		94		11.66 $\pm$ 0.47

\* Calculation of the PLN ratio was described supra.

\*\* Not evaluated.

+ Percent inhibition at 100 nM.

++ Rapamycin had an IC<sub>50</sub> of 0.2 nM as a control for Example 1 and an IC<sub>50</sub> of 8.7 nM as a control for Example 3.

The results of these standard pharmacological test procedures demonstrate immunosuppressive activity both *in vitro* and *in vivo* for the pyrazoles of this invention and the intermediates thereof. Positive ratios in the LAF and PLN test procedures indicate suppression of T cell proliferation. As a transplanted pinch skin grafts are typically rejected within 6-7 days without the use of an immunosuppressive agent, the increased survival time of the skin graft when treated with the compounds of this invention further demonstrates their utility as immunosuppressive agents.

Based on the results of these standard pharmacological test procedures, the compounds of this invention are useful in the treatment of transplantation rejection such as, heart, kidney, liver, bone marrow, and skin transplants; autoimmune diseases such as, lupus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis, and multiple sclerosis; and diseases of inflammation such as, psoriasis, dermatitis, eczema, seborrhea, inflammatory bowel disease. Because the compounds and their intermediates are structurally related to rapamycin and have a similar immunosuppressive activity profile to rapamycin, the compounds of this invention and the intermediates thereof are considered to have antitumor and antifungal activities.

The compounds of this invention may be administered neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the  
5 finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl  
10 cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic  
15 solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially  
20 containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful  
25 in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous  
30 injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

Preferably, the pharmaceutical composition is in unit dosage form, e.g. as tablets or capsules. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example, packeted powders, vials, ampoules, prefilled  
35 syringes or sachets containing liquids. The unit dosage form can be, for example, a

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capsule or tablet itself, or it can be the appropriate number of any such compositions in package form. The dosage to be used in the treatment must be subjectively determined by the attending physician.

The compounds of this invention may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. The compounds of this invention may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermeable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

In addition, the compounds of this invention may be employed as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1 - 5 percent, preferably 2%, of active compound which may be administered topically.

The following examples illustrate the preparation of a representative compounds of this invention.

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### Example 1

#### 31-Deoxy-31-oxorapamycin

Under anhydrous conditions, a solution of rapamycin (2 g, 2.1 mmole) in 20 mL of dry dichloromethane, is treated with Dess-Martin periodinane (1.06 g, 2.5 mmole) in one portion. The reaction mixture was stirred at room temperature for 2 hours, diluted with dichloromethane and washed with saturated sodium bicarbonate and brine. After drying over Na<sub>2</sub>SO<sub>4</sub>, the solvent is removed in vacuo to yield a yellow foam. Purification by flash chromatography (on silica Merck 60, eluant: hexane-ethyl acetate 1:1) provided 0.650 g of the title compound as a pale yellow solid, which exists as a mixture of tautomers, as determined by HPLC (column: Altex Spherogel TSK 10 7.5mm\*60 cm; gradient elution, ethyl acetate in hexane 30 to 70%, flow rate: 5 mL/min).

5 <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): 1.642 and 1.655 (2s, 3H, CH<sub>3</sub>C=C), 1.79-1.82 (m, 3H, CH<sub>3</sub>C=C), 3.11-3.14 (m, 3H, CH<sub>3</sub>O), 3.31-3.38 (m, 3H, CH<sub>3</sub>O), 3.410-3.411 (15 2s, 3H, CH<sub>3</sub>O).

10 <sup>13</sup>C NMR (CDCl<sub>3</sub>, 400 MHz): 210.8, 209.11, 207.95, 207.61, 207.17, 206.25, 202.089, 98.69, 98.65, 98.59, 97.55, 73.84.

15 MS (neg. ion FAB, m/z): 911 (M)<sup>-</sup>, 880 (M-H-OCH<sub>3</sub>)<sup>-</sup>.

### Example 2

#### 31,42-Dideoxy-31,42-dioxorapamycin

The title compound was obtained as a minor product in the preparation of 31-deoxy-31-oxo rapamycin of Example 1. It was also determined to be a mixture of tautomers by the method described in Example 1.

20 <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.64 (s, 3H, CH<sub>3</sub>C=C), 1.80 and 1.863 (2s, 3H, CH<sub>3</sub>C=C), 3.123 (s, 3H, CH<sub>3</sub>O), 3.33 and 3.36 (s, 3H, CH<sub>3</sub>O), 3.461 (s, 3H, CH<sub>3</sub>O).

25 MS (neg. ion FAB, m/z): 909 (M)<sup>-</sup>, 878 (M-H-OCH<sub>3</sub>)<sup>-</sup>.

Anal. Calc'd for C<sub>51</sub>H<sub>75</sub>NO<sub>13</sub> + 3H<sub>2</sub>O: C, 63.53; H, 8.47; N, 1.45

Found: C, 63.60; H, 7.90; N, 1.36

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### Example 3

#### 33-Deoxy-31-deoxy-31,33-(1-hydrazinyl-2-ylidene) rapamycin

A solution of 31-deoxy-31-oxo Rapamycin of Example 1 (0.50 g, 0.55 mmole) and 85% hydrazine hydrate (0.033 g, 0.55 mmole) in 2 mL of methanol was heated with stirring at 60°C for 2 hours. The solvent was removed in vacuo and the residue was purified by flash chromatography (on silica Merck 60, eluant: ethyl acetate-hexane 2:1) to provide 0.080 g of the title compound as a yellow solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.59 (s, 3H, CH<sub>3</sub>C=C), 1.62 (2s, 3H, CH<sub>3</sub>C=C), 3.11-3.25 (m, 3H, CH<sub>3</sub>O), 3.36-3.42 (m, 3H, CH<sub>3</sub>O), 3.61-3.68 (2s, 3H, CH<sub>3</sub>O).

<sup>13</sup>C NMR (CDCl<sub>3</sub>, 400 MHz): δ 208, 197, 170, 169.9, 169.28, 98.9, 98.6, 98.4, 84.94, 84.36.

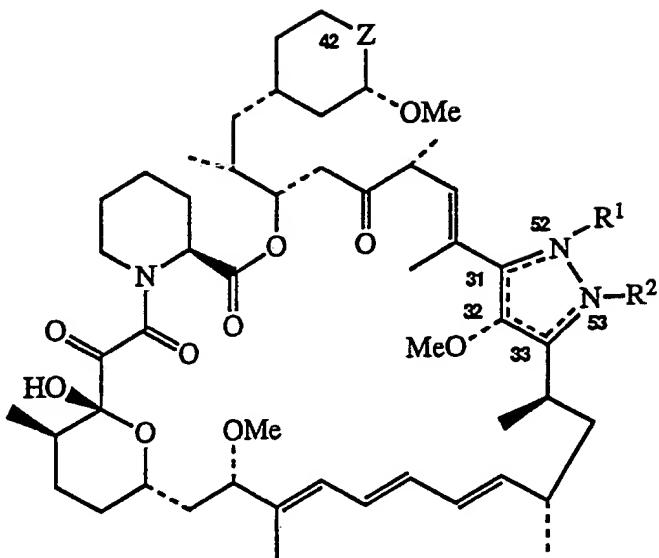
MS (neg. ion FAB, m/z): 907 (M)<sup>-</sup>

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CLAIMS

What is claimed is:

1. A compound of formula



- 5      wherein Z is or ; and
- R<sup>1</sup> is hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms,  
 provided that when R<sup>2</sup> is present, R<sup>1</sup> is absent;
- R<sup>2</sup> is hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms,  
 provided that when R<sup>1</sup> is present, R<sup>2</sup> is absent;
- 10     the dotted lines in the pyrazole ring represent double bonds between the 31- and  
 32-positions and between the 33- and 53-positions when R<sup>1</sup> is present  
 and double bonds between the 31- and 52-positions and between the 32-  
 and 33-positions when R<sup>2</sup> is present;  
 or a pharmaceutically acceptable salt thereof.

- 15    2. A compound of claim 1 wherein Z is or a pharmaceutically  
 acceptable salt thereof.

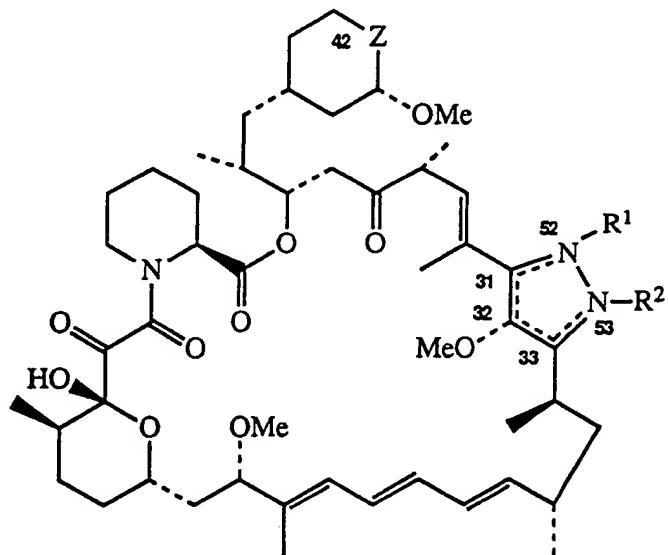
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3. A compound of claim 1 which is 33-deoxo-31-deoxy-31,33-(1-hydrazinyl-2-ylidene) rapamycin or a pharmaceutically acceptable salt thereof.

4. 31-Deoxy-31-oxorapamycin or a pharmaceutically acceptable salt thereof.

5. 31,42- Dideoxy-31,42-dioxorapamycin or a pharmaceutically acceptable salt thereof.

6. A method of treating transplantation rejection, host vs. graft disease, autoimmune diseases, and diseases of inflammation in a mammal by administering to said mammal an immunosuppressive amount of a compound having the formula



10 wherein Z is

or

; and

R<sup>1</sup> is hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms,  
provided that when R<sup>2</sup> is present, R<sup>1</sup> is absent;

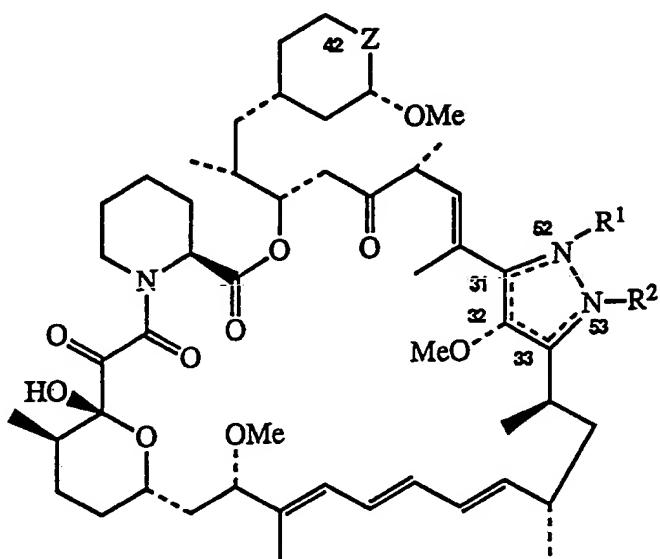
R<sup>2</sup> is hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms,  
provided that when R<sup>1</sup> is present, R<sup>2</sup> is absent;

15 the dotted lines in the pyrazole ring represent double bonds between the 31- and  
32-positions and between the 33- and 53-positions when R<sup>1</sup> is present  
and double bonds between the 31- and 52-positions and between the 32-  
and 33-positions when R<sup>2</sup> is present;

or a pharmaceutically acceptable salt thereof.

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7. A pharmaceutical composition comprising an immunosuppressive amount of a compound having the formula



wherein Z is



5            and  
 $R^1$  is hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms,  
      provided that when  $R^2$  is present,  $R^1$  is absent;

$R^2$  is hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms,  
      provided that when  $R^1$  is present,  $R^2$  is absent;

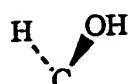
10            the dotted lines in the pyrazole ring represent double bonds between the 31- and  
                32-positions and between the 33- and 53-positions when  $R^1$  is present  
                and double bonds between the 31- and 52-positions and between the 32-  
                and 33-positions when  $R^2$  is present;  
                or a pharmaceutically acceptable salt thereof.

10

15

8. A process for preparing a compound as claimed in claim 1 which comprises one  
      of the following

a) reacting 31-oxorapamycin with hydrazine to give a compound of  
      formula I as defined in claim 1 where Z is



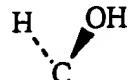
and  $R^1$  or  $R^2$  is hydrogen;

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b) reacting a compound of formula I as claimed in claim 1 wherein R<sup>1</sup> or R<sup>2</sup> is hydrogen with an alkylating agent containing the group R<sup>3</sup> where R<sup>3</sup> is R<sup>1</sup> or R<sup>2</sup> as defined in Claim 1 excepting hydrogen;

or

5 c) oxidizing a compound of formula I as defined in claim 1 wherein Z is



to give a corresponding compound of formula I wherein Z is C=O,  
and if desired after any one of steps a), b) or c) isolating the compound of formula I as  
a pharmaceutically acceptable salt.

10 9. A process for preparing a compound as claimed in claim 4 which comprises  
oxidizing rapamycin using a periodinane oxidizing reagent and if desired isolating the  
compound of claim 4 as a pharmaceutically acceptable salt thereof.

10. A process for preparing a compound as claimed in claim 5 which comprises  
oxidizing rapamycin using a periodinane oxidizing reagent and if desired isolating the  
15 compound of claim 5 as a pharmaceutically acceptable salt thereof.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/09948

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)<sup>6</sup>

According to International Patent Classification (IPC) or to both National Classification and IPC  
 Int.Cl. 5 C07D498/22; A61K31/395; C07D498/18; //C07D498/22,  
 311:00,273:00,231:00,221:00), (C07D498/18,311:00,273:00,

## II. FIELDS SEARCHED

Minimum Documentation Searched<sup>7</sup>

Classification System	Classification Symbols
Int.Cl. 5	C07D ; A61K

Documentation Searched other than Minimum Documentation  
 to the Extent that such Documents are Included in the Fields Searched<sup>8</sup>

III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup>

Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
A	US,A,5 023 263 (G.F. VON BURG) 11 June 1991 see claims 1-7 ---	1-5,7-10
A	EP,A,0 227 355 (UNIVERSITY OF KANSAS) 1 July 1987 see page 1, line 1 - page 4, line 13 & US,A,4 650 803 cited in the application ---	1-5,7-10
A	EP,A,0 323 042 (FISONS) 5 July 1989 see page 4, line 26 - line 27; claims 1,8 -----	1-5,7-10

<sup>6</sup> Special categories of cited documents :<sup>10</sup>

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search

04 MARCH 1993

Date of Mailing of this International Search Report

19.03.93

International Searching Authority

EURPEAN PATENT OFFICE

Signature of Authorized Officer

BRENNAN J.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 92/09948

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim 6 is directed to a method of treatment of the human/animal body, the search has been carried out based on the alleged effects of the compounds.
2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

## Remark on Protest

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT**  
**ON INTERNATIONAL PATENT APPLICATION NO.**

US 9209948  
SA 67296

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
 The members are as contained in the European Patent Office EDP file on  
 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 04/03/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5023263	11-06-91	AU-A-	8162491	13-02-92
		CA-A-	2048540	10-02-92
		EP-A-	0470804	12-02-92
		GB-A-	2246776	12-02-92
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